Remarks

Claims 1, 2, 4-18, 21-26, 29-60, 62, 67-78, and 80-83 remain in the present application. Claims 1, 2, 4-18, 21-26, 29-35, 42-46, 60, 62, 74-78, and 80-83 drawn to the elected invention have been examined on the merits. The Office Action, dated October 12, 2001, has been carefully considered. The claims have now been amended to more clearly set forth the Applicants' contribution to the art and do not introduce new matter into the disclosure of the invention. The basis for the amendments to the claims can be found on pages 21-23, and 47-62 of the specification and further on pages 92-94 of the specification.

New claims 84-90 have been added to better encompass the present invention. The basis for the additional claims can be found on pages 47-62 of the specification.

Summary of Personal Interview with Examiner 37 CFR 1.133.

Applicants thank the Examiner and his Supervisor for meeting with Applicants to discuss the application and Office Action and for their help in suggesting how to amend the present application. It was discussed how the present invention is ***not*** a gene therapy method and that the response is quite different that previous methods, especially those using immune cells. Examiner and his Supervisor suggested possible amendments without specific details, such amendments have now been incorporated into the claims to address concerns brought up in the interview. A final agreement was not reached on the application.

In Response to the Office Action, please consider the following remarks:

The present invention teaches methods for antigen presentation by cells that normally do not carry out that function and to enhance and improve antigen presentation in cells that do carry out that function such as dendritic cells. It teaches generally how to cause an increase in genes important in antigen presentation by artificially introducing sequence non-specific double stranded polynucleotides to cells. Among the objects of the invention is to increase the expression of immune response recognition molecules, and to exploit the expression for the treatment of specific diseases. In this respect, Furuyama *et al.* are very adamant that nonimmune cells do not present antigen and enhance immune responsiveness. Moreover, the references cited as being examples of prior art by exposing cells to polynucleotides are not directed at treating a disease and in most cases refer to situations causing disease.

The instantly claimed invention also teaches that sequence non-specific double-stranded polynucleotides greater than 25 nucleotides in length activate the expression of immune recognition molecules in cells. It teaches a simple and specific system to activate expression of immune Class I and/or Class II molecules of the major histocompatibility complex (MHC), and allows regulation of expression of MHC molecules on the cell-surface of antigen presenting cells and other immune cells.

This invention takes advantage of what is now recognized as an important host defense mechanism. When a nonimmune cell is infected with a virus or bacteria it defends itself by activating or increasing expression of genes which will express self antigens to the immune cell system (Kohn *et al.*). This is a means of attracting immune cells to destroy or neutralize the infection and is termed a bystander immune response. In extreme cases in animals with multiple injections of cells with overexpressed MHC genes and a single self antigen (Shimojo, *et al.*) or in persons with genetic susceptibilities (see Tomer, Shimojo, Kohn) this can lead to autoimmunity. The novel aspect of this work is that the invention recognized that the self defense response could be triggered by ds polynucleotides without a specific sequence and as short as 25 bp entering the cytoplasm of nonimmune cells (Suzuki *et al.*). Moreover, the work recognized that this could be used as a therapeutic tool in appropriate individuals in need of immunotherapy, such as patients with tumor cells needing killing by the immune system (1-8R tumor data) or patients needing adjuvant therapy to produce immune responses (see Iishi paper). The specifics of these uses are discussed extensively as examples.

Objections to the Specification.

The Examiner has indicated that most of the references cited in the IDS filed on March 19, 1999, in Paper No. 5 have not been considered because they were unavailable with the application. It appears that the previously cited references, which were to be attached to the Amendment and Response submitted on July 19 2001, were inadvertently left out of the submission. A supplemental IDS is being sent under separate cover to enter paper copies of the previously cited references.

Rejections Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 1, 2, 4-18, 21-26, 29-35, 42-46, 60, 62, 74-78, and 80-83 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably enable one skilled in the relevant art to make and use the invention commensurate in scope with the claimed invention. The Examiner contends that the claims, while enabling for (a) an *in vitro* method of increasing immune recognition of a mammalian cell by introducing a sequence non-specific double-stranded polynucleotides greater than 25 nucleotides in length into a cell thereby increasing the ability of the cell to present antigen to an immune cell; and is enabled for (b) a method for inducing an autoimmune disease mimicking the human Graves' Disease in a mouse, wherein the method comprises introducing a sequence non-specific double-stranded polynucleotides greater than 25 nucleotides in length into a syngeneic murine cell expressing a functional full-length human thyrotropin receptor *in vitro* and in introducing said murine cell into said mouse; the Examiner contends that the specification does not reasonably provide enablement for other embodiments of the claims.

Applicants respectfully disagree with the Examiner's statement that the claims are not enabled. Applicants' invention teaches a detailed description of how to create an autoimmune disease mimicking Graves' Disease in a mouse model. Applicants teach that this procedure develops an *In vivo* immune response in a mouse system and therefore can be adapted to develop a protective immune responses, which requires only a comparable *in vitro manipulation* to develop an *in vivo* immune response using the in other animal models including humans. They have demonstrated in vivo applicability in a tumor cell model as attested to by Dr. Kohn.

Furthermore, the *in vitro* manipulations needed for the present invention have been fully detailed in references by the Shimojo group (Shimojo, N., Kohn, Y., Yamaguchi, K-I., Kikuoka, S-I., Hoshioka, A., Niimi, H., Hirai, A., Tamura, Y., Saito, Y., Kohn, L. D., and Tahara, K. (1996). Proc. Natl. Acad. Sci. U.S.A. 93:11074-11079; Yamaguchi, K-I., Shimojo, N., Kikuoka, S., Hoshioka, A., Hirai, A., Tahara, K., Kohn, L. D., Kohno, Y., and Niimi, H. (1997) J. Clon. Endocrinol. Metab. 82:4266-4269; and Kikuoka, S., Shimojo, N., Yamaguchi, K-I., Watanabe, Y., Hoshioka, A., Hirai, A., Saito, Y., Tahara, K., Kohn, L.D., Kohno, Y., and Niimi, H. (1998) Endocrinology 139:1891-1989; copies enclosed herewith).

The Shimojo model procedure has also been repeated by the Davies group (Kita, M., Ahmad, L., Marians, R.C., Vlase, H., Unger, P., Graves', P.N., and Davies, T.F. (1999) Endocrinology 140:1392-1398; copy enclosed herewith). The methods of the present invention have been clearly shown to the reproducible and capable of providing an immune response. The issue of its adaptability to be used for treatment of a variety of diseases is evidenced in the tumor remission data and lishi adjuvant response results provided, which were inadvertently missing from previous submission.

The Examiner has noted that the present invention teaches that any double-stranded nucleic acid fragment greater than 25 nucleotides in length introduced into the cytoplasm of non immune cells can induce MHC gene expression directly as well as other essential genes and gene products important for antigen processing in antigen presentation. The effect is sequence independent, is not duplicated by single-stranded nucleic acids, and is different and additive to that of gamma-interferon. The specification further discloses that double-stranded polynucleotides can induce the expression of the 90 K tumor-associated immunostimulator associated with host mechanisms to defend against tumors (Jallal *et al.*, Cancer Res. 55, 3223-3227, 1995).

The Examiner contends that the present teachings cannot be reasonably extrapolated to the broadly claimed invention since this would encompass an *in vivo* method of increasing immune recognition of a mammalian cell as a protective tool by introducing a polynucleotide into a cell ex vivo as a method of increasing presentation of antigen by the cell to achieve a therapeutic immune response.

Applicants respectfully disagree with the Examiner's statement that the claims are not enabled. Applicants respectfully request that the examiner reconsider this in two parts: First, can the invention achieve an immune response *in vivo* that is readily reproduced by scientists skilled in the art and, second, can the immune response be therapeutic.

Applicants' invention teaches a detailed description of how to create an autoimmune disease mimicking Graves' Disease in a mouse model. They use exactly the same procedure used in the Shimojo model and papers adapting the Shimojo model detailed above. No undo or new experimentation was needed to achieve a good immune response. Thus, the applicants respectfully submit that achieving an immune response by *ex vivo* manipulation of the cells with ds polynucleotides is simple and easily performed without undo experimentation, by

simply adapting the Shimojo procedure. They show this can be achieved when an antigen is provided to the cell as a cDNA because this was necessary as a positive control to measure the immune response.

Applicants then evaluated whether this immune response could be protective. They teach that this procedure develops an *in vivo* protective immune response in a mouse system and therefore can be adapted to develop protective immune responses, which require a comparable *in vivo* immune response in other animal models including humans. In support of the Applicant's assertion that the specification is enabled for *in-vivo* protective response, attached is a Rule 1.132 Declaration including additional mouse data generated by the Applicants.

The Examiner has also incorrectly asserted that there is not enough guidance to induce *any and all* autoimmune reactions. Applicants respectfully disagree with the Examiner since the instant invention provides sufficient disclosure that the instantly claimed invention produces a protective response in tumors (the thyroid tumor model) and can generate *in vivo* antibody immune responses. Applicants have practiced the instantly claimed invention and used the identical procedure to express tumor autoantigens that prevent a thyroid tumor from developing or growing as well as a standard injection procedure to develop an antibody response that exceeds a known adjuvant protocol. This research is disclosed in the references attached hereto. This guidance therefore existed and did not require undue experimentation as evidenced by Applicants' own publications. Applicants respectfully submit that because some experimentation may be required, that level of experimentation does not rise to the level of undue experimentation. Applicants have reported this in the reference by Iishi and co-workers (copy attached herewith). The specification provides dosage amounts, frequency of immunization (6 times), route of administration (intraperatoneal or intramuscular) which are described in detail in the Shimojo references (copies attached hereto) and cited on page 94 of the patent application.

The Examiner further asserts that it has been observed that activated immature dendritic cells cannot process certain antigenic peptides mainly of self origin including tumor epitopes to activate the effect or cytotoxic lymphocytes *in vivo*. Applicants respectfully disagree with the Examiner. The article cited by the reviewer concerns work in immune responses largely enhanced by gamma interferon. Van den Eynde states, for example, on

page 151 at the end of his cautionary remarks, that "these ... defective tumor cells start expressing ... when exposed to IFN-γ." The authors admit the defect could therefore be to interferon effects not antigen processing defects. Moreover the authors suggest in their conclusions that whereas processing of immunodominant epitopes is largely acceptable in the interferon model, it is self proteins that are not processed in the [interferon] model. No mention or consideration is given to the ds polynucleotide model, which is additive to and independent of interferon. In the absence of any specific reference to the ds polynucleotide model, this cannot be construed as teaching against according to current rulings. The invention and claims make the point that the ds polynucleotide response is independent and additive to interferon. Further it is contrary to the teachings of the instant invention since dendritic cells are not necessary for the claimed invention.

While the Examiner claims that the nature of the claims would fall within the realm of genetic immunization, Applicants respectfully disagree with the Examiner's remarks concerning DNA vaccines since DNA vaccines work by a very different principle. DNA vaccines are given with known coding sequences since these are the antigen. Thus, the argument in the response regarding the difference between our technology and DNA immunization seems to skirt the fundamental difference that DNA immunization requires the expression of an antigen gene encoded by the administered DNA whereas our technology requires no expression of encoded gene sequences and that no such expression constructs are contemplated or included. Nor does DNA immunization contemplate or anticipate the inherent immunostimulatory effect embodied in every ds oligonucleotide.

With other agents, including CpG motifs, these are given *in vivo* to produce an immune response. The activity of CpG motifs is restricted to immune cells as amply documented in the text of the proposal. In the present case, the polynucleotide is given to cells *in vitro* and allows these cells to turn into antigen presenting cells having an amplification processing of an internal protein or self antigen. This is a critical difference from previous uses. For example, DNA vaccines involve giving simultaneously the coding sequence of a defined antigen. Many such immune responses are dependent on the antigen, most are dominant antigens, whereas therapeutic responses to self antigens usually involve cryptic antigens, which are often unpredictable. In the present claimed invention, the cell itself is allowed to select the cryptic self antigen, present it in context of MHC genes and APC response amplifier. The user does not make the choice of appropriate antigen.

The Examiner has asserted that claims encompassing an *in vivo* method fall within the realm of genetic immunization, which at the effective filing date of the present application was still immature and highly unpredictable. These *in vivo* results couple DNA with an antigen the experimenters felt would be important therapeutically. In most cases this was an assumption wherein the error could well have been the antigen itself not the immunization with double-stranded polynucleotide.

Moreover, we respectfully suggest another incorrectness of the Examiner's assertion that the present invention is unlikely to give a good immune response in humans. This is actually illustrated in the publications on gene therapy cited in the Office Action. Gene therapy involves immunization with a plasmid or viral construct in most cases, *i.e.* double-stranded polynucleotide. Currently, gene therapy does not work because patients develop an immune response to the plasmid antigen. Most research on gene therapy today presumes they can engineer the viral DNA to eliminate this problem. The present patent application provides evidence showing this is not necessarily the case. Thus, hitherto unknown before the present invention, the double-stranded DNA will likely *always* generate an immune response. Further, the Examiner accepts the fact that the Applicants can do this to generate a pathologic response but not a protective response. A protective response is exactly the aim in gene therapy and is successful until the host defense immune system reacts against the foreign polynucleotide. Thus, we respectfully suggest that the Examiner's assertion that it is unlikely that Applicants can generate an immune response is incorrect. The issue then becomes is there sufficient disclosure in Applicants invention to enable a protective immune response. Applicants submit that this is so. Applicants have practiced the invention using the thyroid tumor regression model and have shown that the autoimmune response is by definition protective not pathologic.

The thyroid tumor model illustrates the second aspect of why the Examiner's assertion is incorrect and the cited Chattergoon reference is irrelevant. The present invention is not a DNA vaccine. It is well recognized that critical autoantigens are cryptic. Thus many protective responses do not occur because the antigen that is important to develop the protective response is cryptic and unknown. In the thyroid tumor model Applicants allow the tumor itself to select the array of autoantigens it will present to immune cells. Applicants respectfully submit that this will allow cells to present the cryptic autoantigen generating an

autoimmune response and this is a normal host defense process that we can take advantage of therapeutically.

Applicants further submit that in the Graves' model, the TSHR is a known autoantigen causing an immune response. The selection of the TSHR, which is a known autoantigen, is not a random choice. Thus, using the same immunization proccdure plus thyroid peroxidase (TPO), the Rapoport group generated autoantibodies to TPO that for the first time mimicked the properties of autoantibodies to TPO in autoimmune thyroiditis (Hashimoto's disease). (Jaume, J.C., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S.M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657; copy enclosed herewith). More importantly, it emphasizes the point that the use of a known autoantigen can generate an autoimmune response. In tumors, Applicants do not know the identity of the autoantigen. That is the basis for failures of most studies attempting to develop an autoimmune response against tumors, *i.e.* immune therapy. In the current model, Applicants do not guess the identity of the important autoantigen. Applicants respectfully submit that the instant invention discloses that the cell itself could do this as a normal protective response. Thus, contrary to the Examiner's assertion, undue experimentation will not be required to develop an autoimmune response.

The Examiner further states that results in animal systems are not predictive of outcome in applications to other species or humans citing Ledley as an example. Applicants respectfully submit that the quote from Ledley is taken out of context. Certainly animal experiments are not 100% predictive of what will occur in humans but animal experimcnts are required before human experimentation is commenced. While FDA clinical data is clearly not a requirement for patentability, the FDA considers animal experiments a predictive requirement, *i.e.* a positive result in animal experiments is required before such human experiments can be carried out. We respectfully submit that the contrary argument taken to its extreme, that animal experiments should not be done because they are nonpredictive, is an unacceptable situation for human testing. We respect the possibility animal experiments are not predictable but use of an agent in their absence would be unethical in this day and age.

Ledley discloses virus targeting and suggests that animal experiments are not predictive because the animals do not havc the viral attachment site. In no way does Ledley

otherwise negate the importance of animal models. Thus, animal experiments if possible are often predictive of success in humans. Moreover, Ledley discussion limited to viral receptor or replication determinants not in murine models. He did not extrapolate to all. Certainly, the present case animal model mimics human disease. It is clear in these models as noted in Jaume, J. C., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S. M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657, showing that for first time get antibodies mimicking those in human disease whereas *in vitro* immunization by many failed. It is therefore reasonable to predict this model more likely to succeed and that it pushes predictability to a positive state.

In the present invention, Applicants use a ds polynucleotide and have shown it works in many mouse and human cells, both primary cells and cells in continuous culture. See pages 50-52 of the specification. This argues strongly that this will be useful in human as well as mouse systems. To argue against that is nonscientific. The essence of the scientific process is to provide step-wise experimentation. If an experiment works in a mouse cell, it must be tested in a human cell to extrapolate results. This was done. If an experiment was done *in vitro*, it must also be tested *in vivo* to make the extrapolation. The Examiner appears to suggest that phase III clinical trials in humans must be successfully conducted in humans before an application for a patent can be filed in the United States Patent and Trademark Office. Applicants respectfully suggest that this an unreasonable burden and that this is not the law. Applicants respectfully suggest that the Examiner has placed an unreasonable burden on Applicants that they must prove that the invention is applicable in any and all subjects, when the Applicants have shown it is a likely and reasonable response in which appropriate predictive experiments have been performed. Applicants have disclosed that the invention is positive in multiple cells, human as well as mouse, that the invention works *in vivo* to product an autoimmune disease, and the autoimmune response is a host protective mechanism.

The Examiner asserts that specification fails to provide any guidance regarding delivery to a specific target cell. Applicants respectfully submit that the Examiner is incorrect. The main independent claims of the present invention have now been amended to provide a more clear indication that the double-stranded polynucleotide is provided *ex vivo* to the cells and then the treated cells are administered to a patient. In other words, in the present invention, modified cells are created *in vitro* and these treated cells, when provided to the host, do not need to be targeted. Rather, immune cells in the host are allowed to target to

these newly created "antigen presenting cells" . In essence, the host immune system does the targeting not the agent introduced into a host. Therefore, it is believed that the amended claims as now presented are fully enabled for one skilled in the art.

In the instantly claimed invention, Applicants do not target a cell *in vivo*, but rather Applicants treat the cell with the double-stranded DNA *in vitro* (tissue culture), establish that the cell becomes an APC *in vitro* (tissue culture), destroy the cell with mitomycin, then inject the modified cell *in vivo* intraperitoneally in the Graves' or tumor model. Thus, the individual's immune cells are allowed to target to the injected cells. Applicants' invention permits the host defense mechanism to do what it does normally to protect the organism. The increased class I removes the suppressive CD8 population, the CD4 population attacks, and an autoimmune response to an autoantigen in the injected cells is initiated. In the case of a vaccine, the analogous point holds true, as the vaccine is injected into muscle cells, these become APCs, they initiate the bystander immune response, and immune cell activation is unleashed to create antibodies to protect the organism. Applicants used ovalbumin in the Iishi reference, but this is readily done with tetanus toxin; the ovalbumin is a convenient analog. This is demonstrated in our earlier patents where antitetanus and antiovalbumin responses were measured. See, Singer, D.S., Kohn, L.D. Mozes, E., Saji, M., Weissman, J., Napolitano, G., Ledley, F.D.: Methohds for assessing the ability of a candidate drug to suppress MHC class I expression. U.S. Patent 5,556.754, Feb. 17, 1996; and Singer, D.S., Kohn, L.D. Mozes, E., Saji, M., Weissman, J., Napolitano, G., Ledley, F.D.: Methods of treating autoimmune disease and transplant rejection. U.S. Patent 5,871,950 Feb. 16, 1999. Applicants submit that the ovalbumin response is widely accepted as predictive of a tetanus response.

Thus, Applicants respectfully submit that the Examiner incorrectly asserts that since the prior art does not provide such guidance nor the instant specification supply such teaching, it would therefore have required undue experimentation without a predictable expectation of success for one skilled in the art to make or use the claimed invention.

The Examiner asserted that the specification fails to provide teachings to claims drawn to an *ex vivo* method regarding the following issues:

> "What is the minimum proportion of cells or tumor cells transfected with a sequence non-specific ds-polynucleotide required to induce an effective

> protective or therapeutic immune response, or a desired autoimmune responses in a host to generate various models for specific autoimmune conditions or diseases?", "To which host tissues to transfected cells home in and how long do they need to stay in the system of the host to induce desired immune response?", "Which route of delivery of these transfected cells is effective to obtain the desired immune responses?", "How stable is the state of activated antigen presenting for cells transfected with a nonsequence specific ds-polynucleotide?"

Applicants respectfully submit that the Examiner incorrectly concludes that it would require undue experimentation for a skilled artisan to make and use the broadly claimed invention.

Concerning the Examiner's question of what is the minimum proportion of cells, Applicants respectfully submit that on page 94 of the specification it discloses that $1x10^7$ cells was effective. As disclosed in the specification, this procedure is a direct adaptation of Shimojo *et al.*, PNAS, 93:11074-11079 (1996) (copy enclosed herewith). The Examiner should also note that in Shimojo *et al.* a minimum number of cells was tested ($1x10^5$ and $1x10^6$) and a $1x10^6$ minimum defined. The tumor protective model uses the same number of cells effectively.

Concerning the Examiner's question of which host tissues do transfected cells home, Applicants respectfully submit that the cells do not target host tissues, but rather they remain intraperitoneal and immune cells target to them. Concerning the Examiner's question as to how long do they need to stay in the system of the host to induce desired immune responses, Applicants submit that on page 94 of the specification it discloses that 6 times every two weeks, *i.e.* 12 weeks provides an effective immune response. This is the same duration of time described for the Shimojo model and tumor model.

Concerning the Examiner's question of how stable is the state of activated antigen, Applicants respectfully submit that this is an irrelevant question in mitomycin killed cells (see page 94 of the specification) and is irrelevant *in vivo*, since the procedure is effective. Applicants submit that the state of activated antigen is sufficiently stable to produce an effective immune response *in vivo* under the procedure described.

Applicants respectfully submit that the instantly claimed invention provides sufficient guidance demonstrating an effective immune response and provide evidence supporting its therapeutic or protective usefulness. The applicants respectfully request that the Examiner recognize that developing an autoimmune response against a tumor tissue is nothing more than developing an autoimmune response that causes destroys a tissue as in Hashimoto's thyroiditis or Type 1 diabetes. The difference is the tissue attacked not the immune response. Applicants show a clear immune response in multiple models, Graves' and now tumor treatment. The Examiner construes Graves' as pathologic not protective. Applicants submit that this is not the case. The pathologic response starts as a protective response to eliminate damaged or viral infected cells. The disease is a pathologic overshoot of the initial protective response. The tumor model is a disease model that is protective. Applicants teach how to create an autoimmune disease killing the tumor or its cells akin to diabetes wherein the islet cells are destroyed. With normal islet cells this kills them and is a disease. With tumor cells the same result is protective. Applicants respectfully submit that the Examiner must recognize that protective and therapeutic responses are relative.

The Examiner asserts that relevant information concerning the co-transfected antigen, promoter, vector, cell dosage used, the frequency and route of administering utilized to generate other autoimmune disease models are absent. Applicants respectfully submit that the Examiner's assertion is incorrect. Given the close analogy of the instant invention to the Shimojo model, TPO in thyroiditis and an unknown thyroid tumor antigen in the thyroid tumor model are provided as examples. Applicants respectfully submit that because some experimentation may be required that does not rise to the level of undue experimentation. In addition, given the fact that Applicants teach that the choice of the nucleotide sequence is not critical, the promoter and vector need not be specified - any ds polynucleotide will work in the instantly claimed invention. Cell dose ($1x10^7$) is provided and used in the multiple Shimojo based models upon which the ds DNA Graves' model is based.

The Examiner also asserts that guidance for overcoming differences in anatomy, cell biology, genetics, and immunology between animals is not provided. Applicants respectfully submit that differences in anatomy are irrelevant since intraperitoneal or intramuscular injections are used; this is not gene targeting of specific organs. Differences in cell biology are also irrelevant since multiple cell types and mouse or human cells are shown to have the same ds nucleotide response (see pages 50-52 of the specification). In addition, the

supplemental paper by Molteni enclosed herewith shows the immunologic basis in normal humans.

The Examiner has further asserted that the specification fails to provide teachings to claims drawn to an *ex vivo* method regarding certain claims in that their scope encompasses the use of xenogeneic as well as allogeneic cells transfected with the polynucleotide. The claims have now been amended to provide for the use of homologous cells.

The Examiner incorrectly asserts that the specification fails to provide guidance for the generation of any and all other autoimmune disease models. As pointed out, the Shimojo model has been adapted to produce autoimmune thyroiditis using TPO as an autoantigen. (See: Jaume, J.D., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S.M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657; copy enclosed herewith). The ds DNA model is, in retrospect, similarly effectively replicated in Castoglia, S., Rodien, P., Many, M-C., Ludgate, M., and Vassart, G. (1998) J. Immunol. 160:1458-1465 (copy enclosed herewith) where they injected plasmid plus TSHR. Applicants submit that this model is sufficient to enable one of ordinary skill in the art how to make and use the instantly claimed invention.

With respect to claim 46, the Examiner contends that the specification is not enabled for the method that is claimed. This claim is now been amended to more particularly point out and claim proper scope of the present invention

With regard to claim 77, 78 and 83, the Examiner indicated that the specification fails to teach the parameters involved an increasing or decreasing expression of an antigen. These claims of now been amended to more particularly point out and claim proper scope of the present invention.

In summary, Applicants submit that the Examiner's conclusion that the specification does not provide sufficient direction and guidance for one skilled in the art to make and use the instantly claimed invention appears to be premised on the following: (a) a misunderstanding of the present invention (this is not gene therapy); (b) a misunderstanding of the nature of an autoimmune disease (it is a protective immune response which overcomes self-tolerance and becomes a disease in selected individuals); and (c) a misunderstanding of the clear experimental analogy to the Shimojo model which has been replicated by others and is applicable to other autoimmune responses, not only Graves' Disease. Thus, Applicants respectfully submit that in view of the above mentioned amendments and remarks, Claims 1,

2, 4-18, 21-26, 29-35, 42-46, 60, 62, 74-78, and 80-83, as now amended, are fully enabled and are in condition for allowance.

Rejections Under 35 U.S.C. 112, Second Paragraph

Claims 1, 2, 4-18, 21-26, 29-35, 42-46, 74-78, and 80-82 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that the application regards as the invention. The claims have now been amended herein to address the issues raised by the Examiner as discussed below.

The Examiner indicated that the phrase "thereby activating expression of a gene ... " in claim 1 is unclear. Claim 1 and its dependent claims have now been amended to more clearly set forth the present invention. Additionally, the Examiner indicated that claim one recites a broad recitation "activating expression of a gene or gene and gene product out" in that there is insufficient antecedent basis for certain limitations in the claim or that the metes and bounds of the claim is written cannot be clearly determined. Therefore, claim one and its dependents have now been amended to more clearly set forth the metes and bounds of the claims.

The Examiner stated that claim 2 recites the limitation "the gene or gene product" in that there is insufficient antecedent basis for this limitation. Claim 2 has now been amended to more clearly set forth the metes and bounds of the claims.

The Examiner stated that claim 18 is unclear in one is encompassed by the phrase "the 90 kilodalton tumor associated immune stimulator ..." and what is meant by "intermediate". Claim 18 is now been amended to more clearly set forth the metes and bounds of the claims.

The Examiner states that claims 21, 22, and 26 and the dependent claims of 26 recite the limitation "host organism" and there is insufficient antecedent basis for this limitation. The claims have now been amended to more clearly set forth the metes and bounds of the claims.

The Examiner stated that there is an improper Markush language in claims 46 and 74. The Markush language is now been amended.

The Examiner stated there is insufficient antecedent basis for certain limitations in

claims 34, 35, 45 and 75. The claims have now all been amended to encompass proper antecedent basis for all limitations.

The Examiner has stated that in claim 66 it is unclear what is encompassed by the phrase "the method of treatment is used to enhance another treatment method that enhances an immune response or an antigen presentation." Claim 66 is not within the currently elected invention to be examined on the merits at this time.

The Examiner has stated that claim 76 is incomplete because it lacks a step are steps connecting the steps a-c for treating cancer with a vaccine because there is no step for introducing the vaccine into host with a cancer for treatment purposes. Claim 76 has now been amended to more clearly set forth the metes and bounds of the claims.

The Examiner has stated that claim 80 is unclear as to what is encompassed by the phrase "is coordinate with a treatment with CpG residues." The claim has now been amended to provide a treatment that "is in addition to treatment with CpG motifs."

The Examiner states that claim 77 and 78 or rejected as being incomplete for amending essential steps involved an increasing or decreasing expression of antigen by the cell as recited in step (b). Claims 77 and 78 have now been amended to more clearly set forth the metes and bounds of the claims by providing for a vaccine itself instead and a method for augmenting a vaccine response.

The Examiner has stated that claims 81 and 82 or incomplete because they lack a step are steps connecting the steps a-c for treating a mammalian infectious disease which is associated with immunodeficiency recited in the preamble of the claims because there is no step for introducing the somatic mammalian cell into host. Claims 81 and 82 have now been amended to include a step of introducing the cell into a subject in need of such treatment.

The Examiner has stated that claim 83 lacks a connection between the limitation "the treatment involves activation or maturation of dendritic cells ..." in lines 6-8 and the recited steps (a) and (b). Claim 83 has now been amended to include the further steps of introducing an antigen and the step of introducing the cell into a patient.

In light of the foregoing amendments and remarks, it is submitted that claims 1, 2, 4-18, 21-26, 29-35, 42-46, 74-78, and 80-83 of the present application, as amended herein, are now in form for allowance

Claim Rejections Under 35 U.S.C. 102

The Examiner has rejected claims 1, 2, 4-6, 13, 23, 25, and 44 under 35 U.S.C. 102(b) as being anticipated by Stacey *et al.* (J. Immunol. 157:2116-2122, 1996). The Stacey *et al.* reference teaches that macrophage cells, after ingesting plasmid DNA, are activated leading to the activation of NF-kappa B and induction of a number of inflammatory genes including TNF-alpha. Applicants point out that in the Stacey *et al.* reference, the plasmid DNA is sequence specific and contains CpG motifs. That is, if methylated they lose the effect described above. In the present invention, if the DNA is methylated they do not lose the effect of the present methods. Claims are amended to emphasize this point. Furthermore, the claims have now been amended to provide for introduction of the double-stranded DNA *ex vivo* and the reintroduction of the cells into a subject. Stacey *et al.* further suggests that synthetic double-stranded polyIC RNA but not polyIC DNA similarly activated the cells when incubated with the cells (Fig. 1). The RNA effect cited is the well described polyIC effect wherein when incubated with cells, a receptor is activated and the response is interferon gamma mediated. The polyIC effect herein is not induced by plasma membrane receptor activation but requires insertion into the cytoplasm and is not interferon mediated. Thus, there is no CIITA response and the effect is independent of and additive to interferon gamma. Therefore, it is submitted that the claims as amended are patentable over the Stacey *et al.* reference.

The Examiner has further rejected claims 1, 2, 4, 6, 10, 13, 16, and 74 under 35 U.S.C. 102(b) as being anticipated by Henderson *et al.* (J. Immunol. 159:635-643, 1997). The Henderson *et al.* reference teaches that upon phagocytosis of bacterium *in vitro* by mature human dendritic cells, the dendritic cells are activated in their expression of MHC class I molecules among other molecules. Applicants submit that the methods taught by the Henderson *et al.* reference require specific double-stranded DNA sequences and nonspecific sequence activation is not contemplated at all. Furthermore, the claims have now been amended to provide for selection of cells from a patient in need of immunotherapy, introduction of the double-stranded DNA into cells of a subject *ex vivo* and the reintroduction of the cells into a subject to achieve a therapeutic response. Such therapeutic methods are not contemplated in Henderson *et al.* but simply the study of the mechanism of priming T cells to

a pathogen (last line of introduction). Therefore, it is submitted that the claims as amended are patentable over the Henderson *et al.* reference.

The Examiner has further rejected claims 1, 2, 4, 6, 8, 13, and 42 under 35 U.S.C. 102(b) as being anticipated by Fuller *et al.* (AIDS Research and human retroviruses 10:1433-1441, 1994). The Fuller *et al.* reference teaches a method for the induction of *de novo* antigen production in epidermal cells following article bombardment of an expression vector resulting in MHC-mediated antigen presentation and antibody responses. The Examiner contends that the transfected immune cells in the epidermis must be activated to express MHC molecules in order to present the antigen to achieve an immune response. Applicants respectfully disagree and point out that the activation is dependent upon a specific gene sequence teaching away from the present invention. The methods of the Fuller *et al.* reference describe that the activation by bombardment is actually a response to *tissue injury* to increase specific gene expression. Moreover the Fuller *et al.* reference suggests this is paralleled by changes in gamma interferon suggesting the responses are cytokine mediated. The present invention teaches away from interferon-γ wherein the ds polynucleotide effect is independent and additive to interferon. Furthermore, the claims have now been amended to provide for introduction of the double-stranded DNA into cells of a subject *ex vivo* and the reintroduction of the cells into a subject. Therefore, it is submitted that the claims as amended are patentable over the Fuller *et al.* reference.

The claims 1, 2, 4-6, 8, 10, 13, 16, 23, 25, 42, 44, and 74 have all now been amended to recite the additional steps of obtaining a somatic cell from a subject in need of such treatment and re-introducing the cell into the subject after introduction of the polynucleotide into the cells.

In light of the foregoing amendments and remarks, it is submitted that the claims of the present application, as amended herein, are patentable over the references cited by the Examiner, and it is respectfully requested that the rejection under 35 U.S.C. 102(b) be withdrawn.

Claim Rejections Under 35 U.S.C. 103

The Examiner has rejected claims 1, 8 and 17 under 35 U.S.C. 103(c) and potential 35

U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a). Specifically, the Examiner has rejected claims 1, 8 and 17 under 35 U.S.C. 103(a) as being unpatentable over Stacey *et al.* in view of Brakebusch *et al.* (J. Biol. Chem. 272:3674-3682, 1997; PTO-1449, #15).

The Examiner contends that the Stacey *et al.* reference teaches that bone marrow-derived macrophages ingest bacterial plasmid DNA, *etc.*, and that the cells are then activated leading to activation of a number of inflammatory genes. The Examiner goes on to the state that it is established the double-stranded RNA is also capable of inducing interferons, including β-interferons, and antigen presenting cells such as macrophages. While the Examiner states that the Stacey *et al.* reference does not teach the introduction of double-stranded DNA into the macrophages nor that the expression of MHC molecules are accompanied by increased expression of an about 90 kilodalton tumor-associated immunostimulator, the Examiner states that Brakebusch *et al.* teaches that double-stranded RNA polyIC can induce the expression of 90 kilodalton tumor associated immunostimulator. The Examiner then contends that it would have been obvious to one skilled in the art to have modified the methods taught by Stacey *et al.* by introducing plasmid DNA or double-stranded RNA polyIC to come up with the present invention.

Applicants respectfully disagree with the Examiner. The methods of the present invention are not specific to a DNA sequence and are not a CpG-mediated mechanism as suggested in *Stacey et al.* The mechanism in the present invention is independent of and additive to the mechanism of gamma-interferon. The Brakebusch *et al.* reference emphasizes that interferon and activation of the interferon response element is key to the 90K response. Example 6 shows the ds polynucleotide effect involves an element 3' to the interferon response element and is independent of the interferon action. Although serum levels of 90K do not correlate with killing tumors, 90K overexpression can kill tumors (Jallal *et al.*, 1995, Cancer Research, 55, 3323-3227) and we claim only that it is an apparent intermediate in the immune response that increases MHC class I by the ds polynucleotide. In addition, the methods are capable of activating a nonimmune, somatic cell.

Accordingly, it is submitted that the rejection under 35 U.S.C. 103(a) and (c) is not applicable to the claims of the present invention, as amended herein, and it is respectfully requested that it be withdrawn. The Federal Circuit has held (*In re Sang Su Lee*, 00-1158, Fed. Cir. 2002) that a showing of a suggestion, teaching, or motivation to combine the prior

art references "cannot be dispensed with." The Examiner must advance specific and objective evidence that suggests the claimed combination:

> "the need for specificity .. requires a teaching of a motivation to combine references. The examiner must identify specifically the principle known to one of ordinary skill that suggests the claimed combination."

Neither Stacey *et al.* nor Brakebush *et al.* alludes to the combination or provides specific evidence that suggests it, or contemplates the combination.

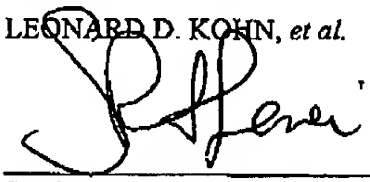
The Applicants respectfully submit this restriction holds for several other references cited as prior art. In most cases, *i.e.* gene therapy injection of plasmid DNA or Viral RNA, DNA vaccines, *etc.*, did not contemplate nonspecific sequence polynucleotide would have these effects. Even CpG motifs contemplate sequence specificity. To argue that one skilled in the art would realize this is not really likely except if he considered or reported the results herein. Further, to combine these observations and the use of a model of creating an autoimmune disease to eliminate a diseased tissue requires a series of assumptions not evident in the references and only capable of contemplation in the light of these data.

In light of the foregoing amendments in remarks, it is submitted that the claims of the present application, as amended here and, are now and form for allowance. Accordingly, early reconsideration and allowance of the claims, as currently pending, are solicited.

The Assistant Commissioner for Patents is authorized to charge any deficiency or credit any overpayment to Frost Brown Todd LLC Deposit Account No. 06-2226.

Respectfully submitted,

LEONARD D. KOHN, *et al.*



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VERSION WITH MARKINGS TO SHOW CHANGES MADE IN THE SPECIFICATION:

IN THE CLAIMS:

Please amend the following claims as indicated:

1. (thrice amended) A method of increasing immune recognition of a mammalian cell [by] in a subject comprising:

(a) obtaining a nonimmune cell from a subject in need of such treatment;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cell and thereby activating expression of a gene[, or gene and] or gene product [that increases immune recognition gene, or gene and gene product, or gene product including MHC class I and class II genes or gene product, peptide processing genes or gene products consisting of TAP-1, TAP-2, a proteosome subunit. class II regulatory genes and gene product's consisting of HLA-DM and invariant chain, co-stimulatory molecule is gene or gene products consisting of B7 co-stimulatory molecule, PKR, IFN-beta, MAP kinase, NF-kB, JAK, and a STATs activation], wherein such activation is involved in antigen presentation, growth, and function of the cell, and which increases the ability of a cell to present antigen to an immune cell; and

(c) re-introducing the cell into the subject.

2. (twice amended) [The method of claim 1 wherein the gene or gene product is derived from the major histocompatibility complex (MHC)] A method of increasing immune recognition of a nonimmune cell in a subject comprising:

(a) obtaining a cell from a subject in need of such treatment;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cell, thereby activating expression of a gene or gene product that increases immune recognition;

(c) introducing an antigen into the cell; and

(d) re-introducing the cell into the subject.

4. (once amended) [The method of claim 1 wherein the double-stranded polynucleotide is derived from a source selected from the group consisting of a bacterium, protozoan, virus, and mammalian cell] A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell and thereby activating expression of a gene or gene product that increases immune recognition, wherein the polynucleotide does not contain a stimulatory CpG motif and wherein such activation is involved in antigen presentation, growth, and function of the cell; and

(c) re-introducing the immune cell into the subject.

5. (once amended) [The method of claim 1 wherein the double-stranded polynucleotide is chemically synthesized without using an enzyme] A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell and thereby activating expression of a gene or gene product that increases immune recognition, wherein the polynucleotide is a noncoding polynucleotide sequence and wherein such activation is involved in antigen presentation, growth, and function of the cell; and

(c) re-introducing the immune cell into the subject.

6. (once amended) The method of claim 1, 2, 4, or 5 wherein [the double-stranded polynucleotide is located in the cytoplasm of the cell] the gene or gene product associated with increased immune activation is selected from the group consisting of MHC class I, MHC class II, TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, RFXA, B7 co-stimulatory molecule, PKR, MAP kinase, NF-kB, JAK, and STAT genes and gene products.

7. (twice amended) [The method of claim 1 wherein the double-stranded polynucleotide is DNA leaking from the cell's nucleus or mitochondria after injuring the cell with an

exogenous or environmental stimulus] A method of increasing immune recognition of a monocyte or dendritic cell within a subject comprising:

(a) obtaining a monocyte or dendritic cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the monocyte or dendritic cell, wherein the polynucleotide does not contain a CpG motif, and thereby activating expression of a gene, or gene product or gene and gene product that increases immune recognition, wherein such activation is involved in antigen presentation, growth, and function of the cell, and which increases the ability of the monocyte or dendritic cell to present antigen to an immune cell of the subject; and

(c) re-introducing the cell into the subject.

8. (once amended) [The method of claim 1 wherein the double-stranded polynucleotide is introduced by transfection microinjection or direct injection using a needle or gene gun] A method of increasing immune recognition of a mammalian immune cell in a subject comprising:

(a) obtaining an immune cell from a subject;

(b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the immune cell, thereby activating expression of a gene or gene product that increases immune recognition, wherein such activation is involved in antigen presentation, growth, and function of the cell, and wherein the polynucleotide contains one or more CpG motifs, which if methylated do not decrease activity of the polynucleotide; and

(c) re-introducing the immune cell into the subject.

9. (once amended) The method of claim 1, 2, 4, 5, 7, or 8 wherein the double-stranded polynucleotide is introduced by the method selected from the group consisting of transfection, microinjection, viral infection of the cell, phagocytosis of a bacterium, virus, or cell, and oncogene transformation.

10. (once amended) The method of claim 1 wherein the cell is a somatic cell [introduction of the double-stranded polynucleotide into the cell occurs by phagocytosis of a bacterium, virus, or cell].

12. (once amended) The method of claim 1, 4, 5, 7, 8, 46, or 60 wherein the cell expresses an autoantigen.

13. (once amended) The method of claim 1 wherein the cell is selected from the group consisting of [non-immune cell, immune cell,] somatic cell, antigen presenting cell and thyroid cell.

14. (once amended) The method of claim 13 wherein the cell is a thyroid cell [is the FRTL-5 thyrocyte].

15. (once amended) The method of claim [2] 7 or 8 wherein [a MHC Class I expression increases greater than a MHC Class II expression as a function of time after introduction of concentration of the double-stranded polynucleotide] the gene or gene product that increases immune recognition is selected from the group consisting of MHC class I and class II genes and gene products, peptide processing genes and gene products, class II regulatory genes and gene products, and co-stimulatory molecule gene and gene products.

16. (once amended) The method of claim [2]6 or 15 wherein [expression of the MHC molecule is measured by determining abundance of MHC protein, MHC transcripts, or MHC gene transcription] the gene or gene product is derived from the major histocompatibility complex (MHC) and wherein a MHC Class I expression increases greater than a MHC Class II expression as a function of time after introduction of concentration of the double-stranded polynucleotide.

18. (once amended) The method of claim 1, 4, 5, 7, or 8 wherein the method further comprises the step of introducing tumor cell RNA into the cell ex vivo [17 wherein the 90 kilodalton tumor-associated immunostimulator is an intermediate in the expression of the MHC class I molecule].

21. (once amended) The method of claim 1, 4, 5, 7, or 8 wherein the cell can induce an autoimmune response when injected into [its host organism] the subject.

22. (once amended) The method of claim 1, 2, 4, 5, 7, or 8 wherein the cell recruits and activates T cells when injected into [its host organism] the subject.

24. (once amended) The method of claim [2]6 or 15 wherein increasing expression of the MHC molecule by double-stranded polynucleotide is additive to and independent of

an interferon-mediated increase in [expression of the MHC molecule] immune recognition.

25. (once amended) The method of claim 1, 4, 5, 7, or 8 wherein the double-stranded polynucleotide is RNA [that increases ß-interferon production by the cell] that is introduced into the cell and does not induce a receptor activated interferon response.

26. (once amended) The method of claim [1] 7 wherein introduction of the double-stranded polynucleotide increases immunogenicity of the cell in a host organism and, further comprising the step of introducing an antigen into the cell prior to introduction of the cell into the subject [, immunizing the host organism with the cell].

29. (twice amended) The method of claim [75]1, 2, 5, 7, 8, or 46 wherein [the polynucleotide is introduced into the cell ex vivo and wherein] the method comprises the further step of treating the cells to prevent cell division prior to introducing the polynucleotide containing cell into a host organism [cell is a mammalian cell].

30. (twice amended) The method of claim [75]1, 2, 4, 5, 7, or 8 wherein neither strand of the polynucleotide encodes [an MHC molecule or a non-MHC] a molecule involved in antigen presentation.

31. (twice amended) The method of claim 1, 2, 4, 5, 7, or 8 wherein the immune system of the subject recognizes one or more antigens presented by the cell [75 wherein increases in expression of the MHC molecule and the non-MHC molecule involved in antigen presentation are measured by determining that the mammalian cell's ability to present antigen is increased].

32. (twice amended) The method of claim 75 wherein [an increase in] expression of both MHC Class I and Class II molecules in or on the [activated APC is measured] cell are increased.

34. (twice amended) The [immunization] method according to claim 74 and further comprising introducing an antigen into the mammalian cell prior to introduction of the activated APC into the subject [host animal].

35. (once amended) The method of claim 34 wherein [immunization] introduction causes an autoimmune reaction in the host animal.

43. (once amended) The method of claim [1] 32 wherein increasing expression of the MHC molecule by double-stranded polynucleotide is additive to or independent of an interferon-mediated increase in expression of the MHC molecule.

45. (once amended) The method of claim 4, 5, or 13 wherein the [immune or antigen presenting] cell is a tumor cell and the [host organism] subject has an increased ability to recognize and kill the tumor cell after such treatment.

46. (twice amended) A method of presenting antigen to the immune system of a mammal in need of immunotherapy comprising;

a) introducing double-stranded polynucleotide into a somatic mammalian cell [with the enhanced ability to present antigen] ex vivo, which improves the ability of the mammalian cell to present antigen; [and]

b) [measuring and increase in] thereby increasing expression of [an MHC] a molecule [or co-stimulatory molecule, or an MHC molecule and co-stimulatory molecule involved in antigen presentation] selected from the group consisting of MHC molecules, TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, [CIITA,] RFXA, B7 co-stimulatory molecule, PKR, [IFN-beta,] MAP kinase, NF-kB, JAK, and STAT; and

c. introducing the somatic cells into the mammal; wherein the cells induce an immune response by the mammal to a antigen.

60. (thrice amended) A method for treating a mammalian disease which is sensitive to immunotherapy which comprises:

a) removing diseased cells from a mammal identified as having a disease which is sensitive to immunotherapy;

b) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the cells;

c) treating the cells to prevent cell division but permit[ing] other metabolic activity; and

d) re-introducing the treated cells into the mammal; wherein the cells induce an immune response by the mammal to a self antigen.

[immunizing the mammal with an effective amount of cells to prevent or alleviate the symptoms of the disease.]

74. (thrice amended) The method of claim 1, 2, or 7 additionally comprising [A method for increasing presentation of antigen by a mammalian cell comprising:

a) introducing a sequence nonspecific double-stranded polynucleotide greater than 25 nucleotides in length into the mammalian cell ex vivo, which causes the cell to have been increased ability to present antigen;

b) increasing the mammalian cells ability to present antigen and] forming an activated antigen presenting cell [at a](APC)[; and

c) measuring an increase in expression of an MHC molecule or a co-stimulatory molecule, or an MHC molecule and a co-stimulatory molecule involved in antigen presentation selected from the group consisting of TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, CIITA, RFXA, B7 co-stimulatory molecule, PKR, IFN-beta, MAP kinase, NF-kB, JAK, and STAT].

75. (once amended) The method of claims 26 or 74 wherein the cell is a tumor cell and [the immunized host organism has an increased ability to recognize and kill the tumor cell] wherein the treatment is in addition to treatment with CpG motifs.

76. (twice amended) [The]A vaccine for treating cancer [with a vaccine,] comprising:

(a) a somatic mammalian cell with the enhanced ability to present antigen to the immune system [comprising;

a) introducing] wherein a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length is introduced into the somatic mammalian cell *ex vivo*, which causes the cell to have an increased ability to present antigen; and

[b) measuring an increase in increasing expression of MHC molecules or co-stimulatory molecules involved in antigen presentation; and [selected from the group consisting of TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, CIITA, RFX5, B7 costimulatory molecule, PKR, IFN-beta, MAP Kinase, NF-κB, JAK, and a STAT; and

c) preparing the mammalian cell for immunization]

(b) a pharmaceutically acceptable carrier.

77. (twice amended) A vaccine [method of] for treating cancer which is sensitive to immunotherapy which comprises;

a) [Removing a diseased cell from a mammal] an adjuvant comprising a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length;

b) an antigen of interest;

[increasing or decreasing the expression of antigen by the cell;] and

c) a pharmaceutically acceptable carrier [immunizing the mammal with an effective amount of the cell to prevent or alleviate the symptoms of the disease].

78. (twice amended) A method for augmenting a vaccine response comprising administering an antigen and an adjuvant to a mammal in need of such treatment, wherein the adjuvant comprises a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length [The method of Claim 77 wherein the method of treatment is used to enhance another treatment method that enhances and immune response or in antigen presentation].

80. (twice amended) The method of claim [76]78 wherein the treatment [involves somatic cells and is coordinate with a] is in addition to treatment with CpG motifs [residues] used to enhance immune cell responsiveness.

81. (once amended) A method for treating cancer which is sensitive to immunotherapy which comprises:

a) obtaining a somatic cell from a subject in need of treatment;

b) introducing a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length into the somatic mammalian cell *ex vivo*, which causes the cell to have an increased ability to present antigen;

[(b) measuring an increase in] c) increasing the expression of [MHC molecules or co-stimulatory] one or more molecules involved in antigen presentation selected from the group consisting of MHC molecules, TAP-1, TAP-2, a proteosome subunit, HLA-

DM, invariant chain, [CIITA,] RFX5, B7 costimulatory molecule, PKR, [IFN-beta,] MAP Kinase, NF-κB, JAK, and a STAT; [and]

[c)] d) preparing the mammalian cell to make suitable for immunization; and

e) introducing the cell into a subject in need of such treatment.

82. (once amended) The method of Claim [80 wherein the disease is AIDS] 81 wherein the polynucleotide is single stranded RNA molecule that, when introduced into the cell, replicates to form a double stranded polynucleotide within the cell.

83. (once amended) A method for treating a patient with a cancer which is sensitive to immunotherapy comprising:

a) removing [diseased] monocytes [cells] from [a mammal] the patient;

c) introducing a sequence non-specific doubled-stranded polynucleotide greater than 25 nucleotides in length into the monocytes *ex vivo*, which causes the monocytes to have an increased ability to present antigen;

c). introducing a tumor cell antigen into the monocytes wherein the antigen is selected from the group consisting of a protein, a peptide, an mRNA encoding antigen and a DNA encoding antigen; and

d). re-introducing the monocytes into the patient

[increasing or decreasing the expression of antigen by the cell; and immunizing the mammal with an effective amount of the cell to prevent or alleviate the symptoms of the disease

wherein the treatment involves activation or maturation fo dendritic cells or peripheral blood macrophages pulsed with antigen in the form of protein, peptide, mRNA encoding antigen, or DNA encoding antigen from tumor cells].

Please add the following new claims:

84. (new) The method of claim 1, 2, 4, 5, 7, or 8 wherein the polynucleotide introduced into the cells is single stranded RNA molecule that, when introduced into the cell, replicates to form a double stranded polynucleotide within the cell.

85. (new) The method of claim 7 wherein methylation of any CpG motifs within the polynucleotide does not effect the activity of the polynucleotide.

86. (new) The method of claim 7 wherein the double-stranded polynucleotide does not contain any stimulatory CpG motifs.

87. (new) The method of claim 76, wherein the cell is a tumor cell.

85. (new) The method of claim 76, wherein the cell is a fibroblast and wherein the method further comprises the step of introducing tumor cell RNA into the cell *ex vivo*.

88. (new) The method of claim 76 or 77, wherein the vaccine is injected in muscle tissue of the mammal.

89. (new) The method of claim 1, 2, 7, or 78 wherein methylation of any CpG motifs within the polynucleotide does not effect the activity of the polynucleotide.

90. (new) The method of 1, 2, 7, or 78 wherein the polynucleotide is a noncoding sequence.

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Art Unit No.: **1632**

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